STUDIES ON THE PERMEABILITY OF THE INTERNAL CYTOPLASM OF ANIMAL AND PLANT CELLS

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STUDIES ON THE PERMEABILITY OF THE INTERNAL CYTOPLASM OF ANIMAL AND PLANT CELLS[1]

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Some form of the plasma membrane theory of permeability is almost universally held at the present time. According to this conception the permeability relations and osmotic properties of cells are due to the presence of surface and vacuolar plasmatic membranes which are of unknown physical and chemical nature. The necessary corollary that the protoplasm proper be freely permeable to all classes of chemicals is generally accepted. Furthermore Pfeffer (1) was forced, by the result of his experiments on Hydrocharis, to assume that a plasmatic membrane is immediately formed on the freshly exposed surface of protoplasm when it comes in contact with water or other media. This view is based upon M. Traube's discovery and investigation of semipermeable precipitation membranes. Pfeffer in particular has shown remarkable ingenuity in the development of the plasma membrane theory but its foundations chiefly rest on indirect evidence, largely from analogy.

The literature is concerned for the most part with the kind of substances which may permeate into cells and the factors involved in permeability.

Overton (2), in his studies on the solubilities of dyes in mixtures of organic solvents and lipoids, assumed that the lipoid

[1] The studies on marine eggs reported in this paper were on during the summer of 1912 at the Marine Biological Laboratory, Woods Hole, Mass., while occupying a table through the courtesy of the Director, Dr. F. R. Lillie, to whom I am indebted for many kindnesses. An abstract of the observations was published in the Biological Bulletin, 1913, xxv, 1.

and not the organic compound was solely responsible for the solution of the dye. The following example shows that this supposition is untenable. Thionin is insoluble in olive oil, benzol and olive oil-lecithin but it is quite soluble in benzol-lecithin. If lecithin were the only factor involved, as asserted by Overton, the thionin should go into solution in olive oil-lecithin. Moreover Loewe (3) found that lipoids do not form true solutions in the organic compounds which were employed by Overton and that dyes are adsorbed by lipoids when added to mixtures of lipoids and organic solvents.

Rhuland (4), Höber (5) are among those who have discussed in detail the factors which favor and prevent the entrance of substances into cells.

Recently Moore and Roaf (6), Meigs (7) and others have questioned the existence of plasmatic membranes; Harvey (8) has made refinements of the indicator method and Evans and Schuleman (9) have materially extended our conception of the factors involved in vital staining with the benzidine dyes. But if future investigations on permeability are to follow sound lines of advance the basic assumptions of the plasma membrane theory must be subjected to experimental study by direct methods. In this paper the results of such a study by means of new methods of technique which permit actual injection of dyes into living cells are given.

MATERIAL AND METHODS

One of the aims of this investigation has been to select species of animals and plants from widely different genera and phyla. The material comprised cells that show great structural and functional differences because it was thought that such cells might show special permeability relationships. The animal material included the eggs of Asterias, Cumingia, Chaetopterus, Nereis, the immature eggs of Necturus, Ameba proteus, Paramecium and striped muscle-cells and epidermal cells of Necturus. The plants used were Saccharomyces, Mucor, Saprolegnia, a number of species of Spyrogyra, Hydrodictyon, the manubrial

cells of Chara, the leaves of Elodea, root-hairs of Vicia faba, Pisum and Hordeum, and the parenchyma cells of Tradescantia.

Barber's intracellular injection method was used for injecting dyes and crystalloids into the interior of cells. Punctures and dissections were made with extremely fine glass needles drawn on the end of Jena glass capillary tubes. For such operations, the cells were mounted in a hanging drop in an open-end moist chamber (10). In some experiments the permeability of the surface layer of marine eggs was modified by the application of very dilute acid, alkali or saponin. The volume of doses was calculated by measuring the diameter of the vacuoles produced. In staining by immersion, both basic and acid dyes were used in closely graded concentrations up to saturated solutions. The length of application was from a few minutes to several hours. In most cases dilute solutions were used, but many cells proved to be impermeable to long applications of saturated solutions of certain of the acid dyes. It may be emphasized that all the observations were made before any evidence of death changes could be detected.

The following dyes were employed. Unless stated to the contrary it is to be understood that they are manufactured by Grüber. A table of the more important solubilities is appended.

THE PERMEABILITY OF CELLS TO BASIC DYES

Janus green (Metz & Co.). (Obtained through the kindness of Prof. R. R. Bensley.) In dilute solution in sea water janus green quickly stained slate blue, granules, about a micron in diameter scattered throughout the cytoplasm of the egg of Asterias. A very small cytoplasmic granule in the egg of Nereis is vitally stained in a few minutes. The whole cytoplasm of the eggs of Cumingia and Chaetopterus was found to contain granules or globules of varying size, which may be stained beautifully by this dye. Granules and the whole cytoplasm of Mucor, Saprolegnia, Paramecium and Ameba are stained vitally by janus green.

New methylene blue G. G. A dilute solution of this dye in sea water stained vitally granules or globules, varying in size

from about 2–10 microns, scattered throughout the cytoplasm of the eggs of Asterias, Cumingia, Chaetopterus and Nereis. The cytoplasm of Paramecium is stained quickly by a dilute solution of the dye in distilled water and the food vacuoles be-

TABLE 1

Basic dyes

	SOLUBLE IN OLIVE OIL	SOLUBLE IN BENZOL	SOLUBLE IN BENZOL-LECITHIN	SOLUBLE OLIVE OIL-LECITHIN
Methylene blue			Quite soluble	
New methylene blue N. (Cassella Color Co.)			Quite soluble	Trace in 24 hours
New methylene blue G. G. (Cassella Color Co.)	Very slightly soluble	Slightly soluble	Very soluble	
New methylene blue R. (Cassella Color Co.)	Very slightly soluble	Slightly soluble	Very soluble	
Toluidin blue	Trace soluble	Trace	Quite soluble	
Pyronin			Quite soluble	
Janus Green	Slight staining of surface		Quite soluble	Trace in 24 hours
Janus Green (Metz & Co.)	Slight staining of surface			Slightly soluble
Methyl Green				
Vesuvin	Slightly soluble		Soluble	
Neutral red	Stains surface	Trace	Quite soluble	
Thionin			Soluble	
Safranin (Water soluble)			Very soluble	Trace

come very distinct. The protoplasm of Mucor and Saprolegnia is beautifully stained vitally by a short application of a dilute solution of New methylene blue G. G.

New methylene blue R. Vital staining of various sized cytoplasmic granules of the marine eggs studied followed the application of a dilute solution of New methylene blue R, in sea water.

TABLE 2

Acid dyes

	SOLUBLE IN OLIVE OIL	SOLUBLE IN OLIVE OIL-LECITHIN	SOLUBLE IN BENZOL
Orange G			
Bleu de Lyon			
Orcein			
Trypan blue			
Trypan red			
Isamin blue			
Eosin		Trace in 24 hours	
Erythrosin		Soluble in 24 hours	Deep staining in 24 hours
Ponceau P. R.			
Aniline blue			
Acid violet			
Nigrosin			
Indigo-carmine			
Biebricher scharlach			
Bordeaux R		Trace in 24 hours	
Aurantia		Trace in 24 hours	Slightly soluble in 24 hours
Indulin			Slightly soluble in 24 hours
Saüregrün			
Solid blue R (Cassella Color Co.)			
Thiocarmine R (Cassella Color Co.)		Soluble in 24 hours	
Acid fuchsin			
Methyl orange		Very slightly soluble	
Congo red			
Methyl red (Kahlbaum)	Soluble	Moderately soluble	
Azolitmin (Kahlbaum)		Trace soluble	
Tropeolin 000 No. 1 (Koenig & Co.)		Soluble	
Sodium alizarin sulphonate (Merck)		Slightly soluble	

The cytoplasm of Paramecium, Ameba proteus, Saprolegnia, Mucor and Spyrogyra was stained blue vitally. Such structural elements as granules and vacuoles were brought out sharply in the living cell.

THE PERMEABILITY OF CELLS TO ACID DYES

. Kuster (11) and Ruhland (4) have recently published accounts of many exceptions to Overton's conclusions regarding the impermeability of cells to acid dyes. By selecting species of animals and plants that have been neglected in most studies on permeability, I have been able to extend as follows, the list of cells permeable to acid dyes:

Trypan blue. The eggs of Nereis and Chaetopterus. The root-hairs of barley and the Windsor bean. Immature Necturus eggs. The peritoneal epithelium of Necturus. Ameba proteus, Paramecium, Mucor and Saprolegnia.

Trypan red. The eggs of Cumingia and Chaetopterus. The root-hairs of barley, the edible pea and the Windsor bean. Ameba proteus, Paramecium, Mucor and Saprolegnia.

Isamin blue. The root-hairs of barley and the Windsor bean.

Aniline blue. The eggs of Chaetopterus. The root-hairs of barley and the Windsor bean, and Mucor.

Acid Fuchsin. The root-hairs of barley and the Windsor bean, and Mucor.

Acid violet. Windsor bean root-hairs. Saprolegnia and Mucor.

Biebricher scarlach. Barley root-hairs. The immature eggs and the peritoneal epithelium of Necturus. Mucor, Saprolegnia and Paramecium.

Indigo-carmine. Saprolegnia and Mucor.

Ponceau P. R. The root-hairs of the Windsor bean. Ameba proteus, Paramecium, Saprolegnia and Mucor.

Nigrosin. Mucor. The peritoneal epithelium and the very immature eggs of Necturus.

Indulin. Root-hairs of Windsor bean. Ameba proteus, Saprolegnia and Mucor.

Eosin. Windsor bean root-hairs, Ameba proteus, Paramecium, Mucor and Saprolegnia.

Erythrosin. Mucor, Saprolegnia and the root-hairs of the edible pea, barley and the Windsor bean. Immature eggs of Necturus.

Bordeaux, R. Paramecium, Mucor, Saprolegnia. The root-hairs of the edible pea and barley.

Aurantia. Mucor, Saprolegnia, Paramecium. The root-hairs of the Windsor bean and the immature eggs of Necturus.

Tropeolin 000 No. 1. Saprolegnia and Paramecium.

THE INTRACELLULAR INJECTION OF DYES

The permeability of the internal portions of the protoplasm to dyes was determined in the following manner. The cells were mounted in a hanging-drop in an open-end moist chamber and the injections were made with very fine capillary pipettes, according to Barber's method.

The eggs of Asterias. A large number of deep intracytoplasmic injections of indigo-carmine, thiocarmine R. trypan blue, methyl red and azolitmin were made. Saturated solutions of the dyes in sea water were used. Small doses varied between about 500 cubic microns and six times that amount.

The general result was the same in all cases. The solution of a given dye formed a cytoplasmic vacuole, out of which the sea water slowly diffused into the surrounding cytoplasm, leaving finally a small mass of precipitated dye to mark the position of the vacuole. In no case did the dye diffuse out of the vacuole into the surrounding cytoplasm. Even in the case of trypan blue, nothing more than an apparent light staining of the wall of the vacuole resulted and this was only obtained twice. The effect of a very slow continuous injection was tried. The capillary pipette was pushed deep into the cytoplasm and a very slow continuous injection made. By this means vacuoles of dye solution of all sizes up to one-fourth of the volume of the egg were produced but without diffusion of the dye into the cytoplasm. Rapid injections of large doses caused the egg to burst and sometimes to separate into several pieces. If the pieces did not undergo an appreciable swelling, no dye diffused into them.

The rate of diffusion of sea water out of cytoplasmic vacuoles is of general biological interest. A vacuole 9 microns in diameter,

filled with a solution of trypan blue in sea water, disappeared in about fifteen minutes, while a vacuole of the same solution twenty microns in diameter required over sixty minutes for complete disappearance of its fluid content.

Ameba proteus. It was determined by experiment that the best results were to be obtained by dissolving the dyes used in about a $\frac{3}{4}$ molar cane sugar solution. Azolitmin, congo red, tropeolin 000, No. 1, sodium alizarin sulphonate, methyl orange, trypan blue and indigo-carmine dissolved in from $\frac{1}{2}$–1 molar cane sugar were injected into the endoplasm of Ameba. The vacuoles formed by the injected dye usually collapsed in a few seconds and the dye rapidly diffused through the endoplasm.

Very small doses of indigo-carmine dissolved in either distilled water or $\frac{1}{2}$ molar cane sugar were injected into the ectoplasm. Vacuoles resulted which usually broke into the endoplasm and disappeared in the course of ten minutes. Neither intraectoplasmic or endoplasmic injections resulted in staining of the ectoplasm.

Striped muscle-cell of Necturus. Indigo-carmine and trypan blue were selected for injection into the striped muscle-cells of Necturus. The dyes were dissolved in 0.8 per cent sodium chloride solution. Indigo-carmine remained entirely localized; trypan blue stained the granular wall of the vacuole and the immediately contiguous muscle substance.

Spyrogyra. A minute drop of a saturated solution of indigo-carmine in distilled water was injected into the sap vacuole of Spyrogyra. In ten minutes the whole protoplasm was blue-green in color. The same experiment was repeated using eosin and erythrosin. A few minutes after injection of either dye, the protoplasm became pink or light red. Vital staining of the cytoplasm and nucleus resulted from the intravacuolar injections of any of the common acid dyes which did not penetrate Spyrogyra when applied to the outer surface.

Some shrinkage of the cytoplasm usually resulted from the puncture of the cellulose wall and the acid dyes, unless they were very dilute, commonly caused the appearance of granules in the cytoplasm.

Hydrodictyon. The whole protoplasm became stained ten to fifteen minutes after the intravacuolar injection of medium sized doses of saturated aqueous solutions of such dyes as indigo-carmine, erythrosin and trypan blue.

THE INTRACELLULAR INJECTION OF CRYSTALLOIDS AND WATER

The permeability of the internal parts of the protoplasm to crystalloids and water was studied by similar methods with the following results:

The eggs of Asterias. The walls of the vacuoles formed by the injection of distilled water and salt solutions were sometimes irregular but the measurements are of sufficient accuracy to be of considerable quantitative value. It was found that a small dose of distilled water diffused into the cytoplasm comparatively slowly. A dose of sea water of the same size required several times as long to disappear; while a vacuole of hypertonic sea water increased in size. It appeared from these results that any part of the cytoplasm might exhibit the same sort of osmotic properties as are shown by the surface. A mature starfish egg was mounted in a hanging-drop. 10,000 cubic microns of distilled water were injected deep into the cytoplasm. At the end of fifteen minutes the vacuole disappeared. Many repetitions of this experiment failed to show any important variations. A 7350 cubic micron dose of sea water was injected into the center of a mature egg. Forty-five minutes later it had disappeared. Repetitions of this experiment brought out no great variation. An 8440 cubic micron dose of slightly hypertonic sea water was injected into the cytoplasm of a starfish egg at 3.30 p.m. At 4.30 the volume was 9500. At 4.40 and at 5 it still remained about 9500 cubic microns in size.

Ameba proteus. Small and large doses of 1 molar sodium chloride, 1 molar potassium nitrate and from $\frac{1}{2}$ to 2 molar cane sugar solution were injected into the endoplasm of Ameba proteus. With the exception of the $1\frac{1}{2}$ molecular cane sugar solution, all the crystalloids injected produced vacuoles which usually collapsed in a few seconds, the vacuolar fluid dif-

fusing through the endoplasm. An injection of very small doses of 0.9 per cent sodium chloride solution was made into the ectoplasm and well defined vacuoles were produced which broke into the endoplasm in the course of a few minutes. The ectoplasm was almost impermeable to a weak sodium chloride solution. Similar experiments were performed with distilled water. The distilled water formed a vacuole in the ectoplasm which showed no appreciable shrinkage in the five to eight minutes which usually intervened between the injection and the breaking of the vacuole into the endoplasm. Small doses of distilled water injected into the endoplasm did not form vacuoles. The water was immediately imbibed by the endoplasm. The injection of large doses, either gave a like result, or the ectoplasm was ruptured and the endoplasm imbibed water so rapidly that the cytoplasm immediately changed into the sol state.

The striped muscle-cell of Necturus. The muscle preparation was made by teasing. A dose of 1200 cubic microns of 9 per cent sodium chloride was injected deep into the sarcoplasm. In fifty minutes the vacuole had about disappeared. Another 1300 cubic micron dose of the same strength sodium chloride required over an hour to disappear. An injection of distilled water took about an hour to be imbibed by the surrounding sarcoplasm. Another very large dose of distilled water did not disappear in one hour and one-half.

When doses of from 1½–2 molar cane sugar were injected the muscle died and became granular in the course of two hours; but little change in the size of the vacuole was observed except in one or two cases where there seemed to be a little increase in size. Exact quantitative measurements could not be made as the muscle-substance was so rigid that the vacuoles were very irregular in outline. As a result it was impossible to make exact measurements for the calculation of the volume of the vacuole. The measurements were exact enough to show that the sarcoplasm of the striped muscle-cell of Necturus is only slightly permeable to water, somwehat less so to 0.9 per cent sodium chloride and still less to strong cane sugar solution.

THE EFFECT OF PUNCTURE ON THE PERMEABILITY OF MARINE EGGS FOR DYES

The eggs of Asterias, Cumingia, Chaetopterus and Nereis were selected for this series of experiments. The permeability of the normal uninjured eggs for concentrated solutions of acid dyes was determined, and only those dyes were used which did not stain the uninjured egg after application lasting from 30 minutes to several hours.

It was determined experimentally that slight incisions and punctures of the cytoplasm cause localized swellings of the nature of concentration gradients. The cytoplasm of such a swollen area increases gradually in concentration from the surface to the interior. A curve of the gradient would start at the surface which would represent the base line and slowly rise, then suddenly steepen and soon become flat. The flat top would represent the normal cytoplasm. The swollen area produced by cutting, or puncture, usually measured between 6 and 30 microns in thickness. The eggs were mounted in a hanging-drop in an open-end moist chamber. A Jena glass needle held in a three-movement pipette holder was used for incising the eggs.

Many variations of these experiments were made. The concentrations and the length of application of the dye and the size of the incisions and punctures were all varied. The dyes were dissolved in sea water. It was necessary in this kind of experiment to use dyes concentrated enough to be visible in small drops.

The purpose of the production of fluidity gradients in marine eggs was to determine whether their permeability relations are due to peculiar surface films, or to the whole protoplasm. If the current plasma-membrane theory of permeability be correct, a dye which penetrates the surfaces of a swollen area of cytoplasm ought to penetrate the whole interior of the egg.

In the majority of cases the dye did not penetrate the surface of the swollen area of cytoplasm. Those dyes which entered the surface of such swollen areas stopped at varying levels in the gradient. In no case was the whole interior of the egg stained.

TABLE 3

Egg of Asterias

DYE	SOLUTION	SWOLLEN AREA IN INCISED CELLS	SWOLLEN AREA IN PUNCTURED CELLS	CONTROL UNINJURED CELLS
Trypan blue	Moderately concentrated			
Trypan red	Moderately concentrated			
Solid blue R	Moderately concentrated	− (40m)	− (40m)	
Azolitmin	Moderately concentrated			
Nigrosin	Moderately concentrated			
Thiocarmine R	Moderately concentrated		+	
Tropeolin 000 No. 1	Moderately concentrated			
Blue de Lyon	Moderately concentrated		− (30m)	
Eosin	Dilute	+ (10m)	+ (10m)	
Erythrosin	Dilute	+	+	
Janus green (Grübler)	Dilute		+	

TABLE 4

Egg of Neresis

DYE	SOLUTION	SWOLLEN AREA IN INCISED CELLS	SWOLLEN AREA IN PUNCTURED CELLS	CONTROL UNINJURED CELLS
Azolitmin	Concentrated	− (30m)		
Trypan red	Moderately concentrated	+	+	
Thiocarmine R	Dilute	− (30m)	− (30m)	

TABLE 5

Egg of Chaetopterus

DYE	SOLUTION	SWOLLEN AREA IN INCISED CELLS	SWOLLEN AREA IN PUNCTURED CELLS	CONTROL UNINJURED CELLS
Solid blue R	Concentrated			
Erythrosin	Concentrated	+ (30m)	+ (30m)	
Eosin	Dilute			
Nigrosin	Concentrated			

TABLE 6

Egg of Cumingia

DYE	SOLUTION	SWOLLEN AREA IN INCISED CELLS	SWOLLEN AREA IN PUNCTURED CELLS	CONTROL UNINJURED CELLS
Trypan blue	Dilute			
	Concentrated	+	+	
Thiocarmine R	Dilute	− (30m)	− (30m)	
Nigrosin	Dilute	+	+	
Solid blue R	Dilute			
Erythrosin	Dilute	+	+	

THE EFFECT OF PUNCTURE OF THE CELL-WALL ON THE PERMEABILITY OF PLANT CELLS FOR ACID DYES

Puncture of the cell-walls of plants has given results that seem to be of signal importance for the general problem of permeability.

Hydrodictyon, Saprolegnia, some five species of Spyrogyra, the leaves of Elodea, the leaf parenchyma of Tradescantia and the manubrial cells of Chara were used in these experiments. The material was mounted in a hanging-drop. The cells walls of most of the species tested were extraordinarily tough and rigid so much so that the Jena glass needles were frequently broken in making the punctures. Both protoplast and cell-wall were punctured in many cases. The acid dyes employed were dissolved in either distilled water or tap water which was neutral to litmus but slightly alkaline to phenothalein. The solution of the dye was always concentrated enough to give a distinct color to microscopical droplets. The chief effect of increasing the concentration of a dye solution was to increase the speed and depth of staining. The dye was applied either before or after puncture of the cell-wall. Briefly stated, puncture of the cell-wall, or the cell-wall and protoplast, made most of the cells permeable to all the common acid dyes which under the same conditions do not penetrate the unpunctured cells. The protoplast was in some cases separated from the cell-wall by slight plasmolysis with about $\frac{1}{2}$ molar cane sugar and the cell-wall was then very carefully cut or punctured without in-

juring the protoplast. In such cases the acid dyes penetrated the cytoplasm and nucleus about as readily as after puncture of the protoplast. If the cells were first thoroughly plasmolyzed and the walls cut acid dyes in quite concentrated solution occasionally stained the outer surface of the shrunken cytoplasm.

TABLE 7

Spyrogyra

DYE	SOLUTION	CELL WALL + PROTOPLAST PUNCTURED	CONTROL NOT PUNCTURED
Erythrosin	Moderately concentrated	+ (5 min.)	
Eosin	Dilute	+ (8 min.)	
Orange G	Moderately concentrated	+ (3 min.)	
Indigo-carmine	Concentrated	+ ((4 min.)	
Trypan blue	Moderately dilute	+ (10 min.)	
Trypan red	Moderately concentrated	+ (3 min.)	
Isamin blue	Saturated	+ (surface stained only) (10 min.)	
Aniline blue	Moderately concentrated	+ (8 min.)	
Nigrosin	Fairly concentrated	+ (10 min.)	
Thiocarmine	Fairly concentrated	+ (20 min.)	
Tropeolin 000 No. 1	Moderately concentrated	+ (10 min.)	(slight)
Methyl orange	Dilute	+ (10 min.)	(slight)
Methyl red	Moderately concentrated	+ (10 min.)	
Methylgrün	Slightly concentrated	+ (15 min.)	
Congo red	Moderately concentrated	+ (10 min.)	
Acid fuchsin	Moderately dilute	+ (4 min.)	
Biebricher Scharlach	Comparatively dilute	+ (5 min.)	
Bordeaux R	Moderately dilute	+ (4 min.)	
Pouceau P. R	Dilute	+ (5 min.)	

It was noted that rapid plasmolysis of spyrogyra is accompanied by a definite softening and loss of rigidity of the cell-wall. This fact was determined by dissecting plasmolyzed cells. Furthermore, it was determined that many acid dyes will enter and stain the whole protoplasm of Spyrogyra cells

if added very soon after recovery from rapid plasmolysis. A mucilaginous substance usually poured out of the cell-wall of all the species of Spyrogyra examined during rapid plasmolysis. This substance can be stained red in neutral or very weakly alkaline solutions of sodium alizarin sulphonate.

Such facts prove conclusively that neither the plasma membrane nor the protoplast, but the cell-wall is responsible for the impermeability of many plant cells for acid dyes.

On account of the length of this series of experiments only selected examples can be given.

TABLE 8

Slightly plasmolysed Spyrogyra

DYE	SOLUTION	PLASMOLYSED WITH PUNCTURED CELL WALL. PLASMA MEMBRANE UNINJURED	CONTROL PLASMOLYSED WITH CELL WALL AND PLASMA MEMBRANE UNINJURED
Biebricher scharlach..	Very dilute	+ (8 min.)	
Bordeaux R.....	Very dilute	+ (2 min.)	+ (8 min.)
Erythrosin.....	Dilute	+ (5 min.)	+ (30 min.)
Ponceau.....	Dilute	+ (5 min.)	+ (30 min.)
Indigo-carmine.....	Dilute	+ (5 min.)	+ (15 min.)

TABLE 9

Plasmolysed Spyrogyra allowed to recover

DYE	SOLUTION	AFTER RECOVERY FROM PLASMOLYSIS	CONTROL NOT PLASMOLYSED
Biebricher scharlach.....	Moderately concentrated	+ (4 min.)	
Saure violet.....	Moderately concentrated	+ (3 min.)	
Bordeaux R.....	Moderately concentrated	+ (8 min.)	
Indigo-carmine.....	Moderately concentrated	+ (15 min.)	
Aniline blue.....	Moderately concentrated	+ (10 min.)	

THE EFFECT OF ACIDS, ALKALI, AND SAPONIN ON THE PERMEABILITY OF MARINE EGGS

It was determined by experiment that fluidity gradients could be produced in the cortex of marine eggs, by the application of very weak acids, alkali and saponin. The amount of swelling was controlled by direct observation. The eggs of Chaetop-

terus, Cumingia, Nereis and Asterias were chosen for this experiment on account of their large size and favorable optical properties.

Unfertilized marine eggs which were allowed to lie in sea water, underwent cortical swelling in from six to thirty-six hours. Such changes are usually interpreted as autolytic. This is another method for establishing fluidity gradients in marine eggs. The results of all the methods were essentially the same. Acid dyes were selected which did not penetrate normal marine eggs even after long applications of concentrated solutions. The eggs were treated with very dilute acid, alkali, or saponin or allowed to autolyze until a definite cortical swelling occurred; then the eggs were washed in several changes of fresh water and solutions of the acid dyes added. Some of the dyes did not penetrate the surface of the swollen cortex; others entered to the depth from 3 to 8 microns; and still others as far as 15 microns, or the beginning of the unswollen cytoplasm. It is self evident that if a plasma-film prevents the entrance of these acid dyes and if the dyes once pass beyond the surface-film, the whole cytoplasm ought to be immediately stained; but the dyes are actually stopped at different levels of the gradient. An extract from my protocols will serve to make this clear.

Eight drops of 1 per cent solution of saponin in sea water were added to 20 cc. of sea water containing fresh Nereis eggs. In five minutes sufficient swelling occurred to establish the desired fluidity gradients in the cortex.

A five minute application of a moderately concentrated solution of erythrosin in sea water resulted in a pink to deep red staining of the cortex. The interior of the egg remained unstained twenty-five minutes later. The cortical swelling was commonly very unequal and some of the eggs showed general cytoplasmic swelling, being twice their normal diameter. Such eggs may become red throughout in thirteen minutes.

Nigrosin. A moderately concentrated solution of nigrosin in twenty minutes only stained the surface of eggs showing very definite cortical swellings.